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CHEMOTHERAPY OF MALARIA

ANNUAL SUMMARY REPORT

DORA S. RANE

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Dr. Leo Rane Research Laboratory
University of Miami
Miami, Florida 33142

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P. berghei blood-induced malaria infection in mice - based on mortality.

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Primary screen - quantitative evaluation of potential antimalarial activity.

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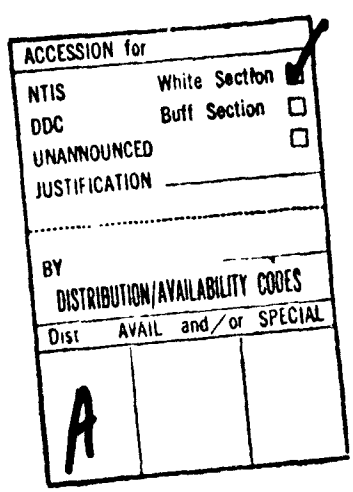
Foreword

In conducting the research described in this report, the investigator(s) adhered to the "Guide for Laboratory Animal Facilities and Care", as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences-National Research Council.



I. CONTINUATION OF THE SCREENING PROCEDURE FOR THE EVALUATION OF ANTIMALARIAL ACTIVITY OF CANDIDATE COMPOUNDS USING PLASMODIUM BERGHEI INFECTIONS IN MICE

This antimalarial screening program was initiated after the discovery that the Plasmodium falciparum parasite chiefly responsible for the high incidence of malaria in Vietnam was resistant to drugs such as chloroquine and quinine, generally recognized since World War II as satisfactory antimalarial agents. The urgent need for the screening operation was further emphasized when resistant strains were encountered in different malaria centers in Asia and in South America.

A total of 246,741 compounds were tested from December 1, 1961, through May 31, 1975.

Table I summarizes the compounds tested and the mice used from December 1, 1961, through May 31, 1975.

The test system designed specifically for this operation is based on blood-induced Plasmodium berghei malaria infections in mice. It is a relatively simple and fast procedure. Its assessments of antimalarial effect and host toxicity are reproducible and reliable. It has introduced a new efficient method for screening large numbers of candidate compounds that have been successfully used as a model in the development of other large-scale screening operations.*

All compounds evaluated were obtained from the Department of Medicinal Chemistry at the Walter Reed Army Institute of Research and included:

(1) compounds structurally related to chemicals of known value as antimalarial agents;

(2) compounds structurally unrelated to compounds known to have antimalarial activity;

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(1) A screening operation using blood-induced P. gallinaceum infections in chicks.
(2) A screening operation using sporozoite-induced P. gallinaceum infections in chicks.
(3) A screening operation based on blood-induced Trypanosoma rhodesiense infections in mice.

(3) structural analogues of compounds found active in our test system and representing several novel chemical groups.

Our own breeding colony of ICR/HA Swiss mice has continued to supply the animals used in our tests.

Evaluations of activity have been based on the responses to candidate compounds by P. berghei malaria in mice as expressed in comparisons of the maximum survival time of treated malaria-infected animals and the survival time of untreated malaria-infected controls.

Using young ICR/HA Swiss mice and a standard inoculum of P. berghei, it has been possible to produce a consistently uniform disease fatal to 100% of untreated animals within 6 to 7 days.

Since an established disease is less responsive to treatment than a disease in the early stages of development, treatment is withheld deliberately until a fairly high degree of parasitemia is evident. Test compounds are administered parenterally in a single dose on the third day post-infection at which time a 10-15% parasitemia has developed.

To be classified as active, a compound must suppress the disease and produce an unquestionably significant increase, 100% or more, in the life span of the treated animals over that of the untreated controls.

The severity of the challenges set up in our test system enhances the reliability of our evaluations and the antimalarial potential of the compounds selected for intensive preclinical studies.

METHOD*

ANIMAL HOSTS. The total supply of animals needed to screen candidate compounds has been obtained from our own breeding colony of ICR/HA Swiss mice. Test animals weigh from 18 to 20 grams, weight variations in any given experimental or control group being carefully limited to 2-3 grams. In any given test all animals are of a single sex and approximately the same age.

*Designed, developed and operated by Dr. Leo Rane until 1973, then operated by Mrs. Dora Rane until 1976.

P. berghei malaria in mice.

TABLE I

SUMMARY OF SCREENING LEVELS

DECEMBER, 1961 - NOVEMBER, 1975

	NUMBER OF COMPOUNDS	NUMBER OF MICE
DECEMBER, 1961 - MAY, 1964	6,915	250,000*
JUNE, 1964 - MAY, 1965	13,114	215,715
JUNE, 1965 - MAY, 1966	22,731	350,449
JUNE, 1966 - MAY, 1967	34,093	531,200
JUNE, 1967 - MAY, 1968	40,465	636,525
JUNE, 1968 - MAY, 1969	38,150	603,225
JUNE, 1969 - MAY, 1970	22,376	411,270
JUNE, 1970 - MAY, 1971	18,108	322,140
JUNE, 1971 - MAY, 1972	14,874	262,245
JUNE, 1972 - MAY, 1973	14,276	231,450
JUNE, 1973 - MAY, 1974	11,035	168,664
JUNE, 1974 - MAY, 1975	10,604	168,725
TOTAL	246,741	4,151,608

*Includes mice used in the development of the test.

Animals on test are housed in metal-topped plastic cages, fed a standard laboratory diet and given water ad lib. Once the infected mice are given the drug they are placed in a room maintained at 84° F (± 2° F) and a relative humidity of 66% (± 2%).

TEST PROCEDURE. Test animals receive an intraperitoneal injection of 0.5 cc of a 1:244 dilution of heparinized heart blood with a minimum of 90% parasitized cells, drawn from donor mice infected four days earlier with P. berghei. The donor strain is maintained by passing every four days in separate groups of mice inoculated with 0.5 cc of a 1:50 dilution of heparinized heart blood.

To check factors such as changes in the infectivity of our P. berghei strain or in the susceptibility of the host one group of mice is infected but not treated which serves as the negative control. In order to determine the effect a drug exerts on a malarial infection two parameters are measured; the first being an increase in survival time, and the second concerns curative action. For comparative purposes one standard compound, pyrimethamine, is administered at one level (120 mg/kg) to a group of 20 mice which serves as a positive control producing definite increases in survival time and curative effects. Another function of the positive control involves monitoring three procedures; the drug weighing, the preparation of drug solutions and suspensions, and the administration of drugs.

DRUG ADMINISTRATION. Test compounds are dissolved or suspended in peanut oil before they are administered.

Treatment consists of a single dose given subcutaneously 3 days post-infection. At the time of treatment a 10-15% parasitemia has developed. Although the disease is well established, it has not yet caused sufficient debility to affect an evaluation of the test compound's toxicity.

Deaths that occur before the 6th day, when untreated controls begin to die, are regarded as the result of a compound's toxic effects and not as the result of action by the infecting parasite.

In each experiment the compound on test is administered in graded doses (640, 160 and 40 mg/kg) to groups of 5 mice per dosage level. Increases in the dose levels of highly active compounds usually are followed by increases in the survival time of the treated mice.

If an active drug is toxic for the host, the toxicity of this compound may become a limiting factor to changes in dose levels.

Treated animals alive at the end of 60 days are considered as cured.

DRUG ACTIVITY. Acceptance of a drug as being sufficiently active for detailed studies is predicated on the margin between the maximum tolerated dose (MTD) and the minimum dose producing a significant effect (MED). A maximum tolerated dose is defined as the highest dose causing no more than one of five animals to die. The minimum effective dose is defined as the minimum dose increasing the life span of treated animals by 100% over the life span of untreated controls.

An increase of 100% in survival time is considered the minimum significantly effective response for a candidate compound.

Clearly inactive compounds are rejected after one test, borderline compounds after two tests. Active compounds are subject to a test to determine a dose-response curve (a 6 or 9 dosage level test) so that the spread between the maximum tolerated dose (MTD) and the minimum dose producing a significant effect (MED) may be established. The total number of active compounds from June 1, 1970, to May 31, 1975 is summarized in Table II.

P. berghei malaria in mice

TABLE II

SUMMARY OF ACTIVE COMPOUNDS

JUNE 1, 1970 - NOVEMBER 21, 1975

YEAR	NUMBER OF COMPOUNDS TESTED	NUMBER OF COMPOUNDS ACTIVE
JUNE 1, 1970 - MAY 31, 1971	18,108	805
JUNE 1, 1971 - MAY 31, 1972	14,874	593
JUNE 1, 1972 - MAY 31, 1973	14,276	771
JUNE 1, 1973 - MAY 31, 1974	11,035	394
JUNE 1, 1974 - MAY 31, 1975	10,604	616
TOTAL	68,897	3,179

II. SECONDARY SCREENING*

Twenty-nine new compounds were tested against the drug-sensitive P-line of Plasmodium berghei. Of the 29 tested 17 exhibited suppressive activity equal to or greater than quinine. The remaining 12 compounds were either inactive or had activity less than quinine. One compound, WR 152,520, was tested against the C, S, T and M-lines and exhibited no cross resistance with chloroquine, dapsone, cycloguanil, or pyrimethamine.

Out of the 17 good compounds 7 exhibited quinine equivalent values of greater than 25. These 7 compounds are in the following groups:

CHEMICAL GROUP	NUMBER OF COMPOUNDS	WR NUMBER	BOTTLE NUMBER
Quinolinemethanols	2	29,252	BD 71301
		213,471	BE 13920
Orotic acid	1	152,520	BC 52936
Phenanthrenemethanol	1	181,020	BD 71258
Methoxyquinaldine	1	182,234	BE 10198
Tetrahydroquinazoline	1	214,767	BE 15335
Cinchona alkaloid	1	215,440	BE 22385

Several of the compounds developed and tested in the last several years were also tested. These were WR 33,090, 33,063, 122,455, 142,490, 158,122, and 180,872. All except the two thioquinazolines (158,122 and 180,872) and one phenanthrenemethanol (122,455) were tested against two or more resistant lines. Each compound exhibited approximately the same degree of suppressive activity as noted in previous testing in Georgia.

Seven older reference compounds were tested such as quinacrine, quinine, amodiaquine, sulfalene, cycloguanil pamoate, sulfadiazine, and a guanylhydrazone. These drugs exhibited a similar degree of suppressive activity as they did in the Georgia test system except for sulfalene and sulfadiazine which had slightly less suppressive activity.

The four main drug-resistant lines of P. berghei (C, S, T, and M) were tested to ascertain their degree of resistance and cross resistance to each of the four main drugs used to treat malaria in the world today (chloroquine, dapsone, cycloguanil and pyrimethamine). The chloroquine-resistant C-line is completely resistant to chloroquine while completely susceptible to dapsone, cycloguanil and pyrimethamine. The dapsone-resistant S-line is completely resistant to dapsone while

*June 1, 1974 - March 15, 1975.

completely susceptible to chloroquine, cycloguanil and pyrimethamine. The cycloguanil-resistant T-line is completely resistant to cycloguanil and almost completely resistant to pyrimethamine while it shows no resistance to chloroquine or dapsone. The pyrimethamine-resistant M-line is completely resistant to pyrimethamine and cycloguanil while completely susceptible to chloroquine and dapsone.

Two new drug-resistant lines of P. berghei were added to the battery of existing lines. One resistant to quinine and the other resistant to a quinolinemethanol (WR 142,490). Both lines are completely resistant to their respective compounds. Several compounds were tested against each line.

A special test was performed to determine whether WR 180,872 or its dextrorotatory isomer (WR 214,819), or its levorotatory isomer (WR 214,767) was superior in suppressing drug-sensitive P. berghei. Each compound was mixed in peanut oil and hydroxyethyl-cellulose-tween 80 then administered via three different routes (orally, intraperitoneally, and subcutaneously). The data indicated the best suspending agent was peanut oil and the best route of administration was the I.P. route for the two active compounds WR 180,872 and WR 214,767. WR 214,819 was not active. WR 214,767 had almost twice the Q value as WR 180,872 when both were suspended in peanut oil and administered via the I.P. route.

Previous testing with quinine has shown the suppressive effects of 50 and 100 mg/kg/day levels are not uniformly reproducible so a special experiment was designed to try and solubilize quinine. By comparing the SD_{90} values obtained for each of the differently mixed suspensions of quinine no definite conclusions were reached as to which method was best. Because of this discrepancy chloroquine was substituted for quinine as the positive control and a quinine equivalent value is still calculated for each new compound based on using a conversion factor and the SD_{90} value obtained with chloroquine.

Four drug combination tests were done to detect any possible synergistic suppressive activity. In one experiment WR 180,872 and WR 158,122 were administered subcutaneously in 2:1 and 1:1 ratios respectively. The data indicated possible synergistic activity in the 2:1 ratio while an additive effect was observed with the 1:1 ratio. In the second experiment the following combinations and results were obtained.

DRUGS	RATIO	SYNERGISM
2,978:7,557	1:1	Yes
33,063:7,557	10:1	Possible
33,063:2,978	10:1	Yes

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